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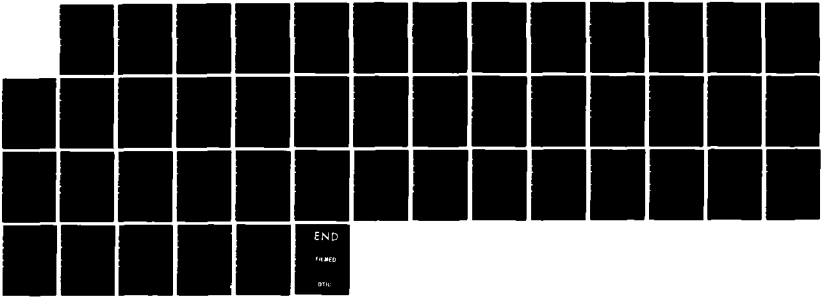

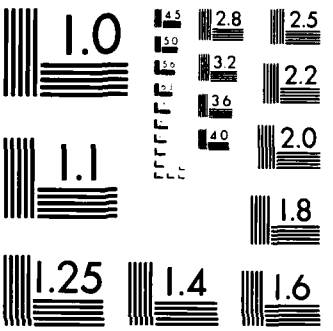

PULMONARY ULTRASTRUCTURAL CHANGES IN SEPSIS

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PREFACE

Research work on pulmonary ultrastructural changes performed with the support of ONR Contract #N00014-79-C-0425 is presented in the attached manuscripts:

Chaudhuri, B., Moss, G., and Das Gupta, T.K., "Pulmonary Ultrastructural Changes in Endogenous Septic Shock." In preparation.

Olson, L., Moss, G., Baukus, O., and Das Gupta, T.K., "The Role of C5 in Septic Lung Injury." Accepted for publication in Annals of Surgery.

#1

PULMONARY ULTRASTRUCTURAL CHANGES IN ENDOGENOUS SEPTIC SHOCK

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RUNNING HEAD: ENDOGENOUS SEPTIC SHOCK

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ABSTRACT

Sepsis and septic shock are associated with lung injury and Adult Respiratory Distress Syndrome, but the pulmonary ultrastructural changes seen in this setting have not been well described. In the following study, thirty rats were subjected to lethal endogenous septicemia by the cecal ligation-puncture technique. A similar number of control animals underwent laparatomy without cecal ligation. After 48 hours, five surviving rats in each group were killed and their lungs studied by electron microscopy including morphometric analysis. The principal findings included diffuse capillary thrombosis; hyperactive appearing granular pneumocytes plus free alveolar surfactant; increased number of interstitial white blood cells and alveolar macrophages plus decreased number of white blood cells within capillaries. These studies suggest that septic shock produces injury to all compartments of the lung.

Key Words: Septic Shock
Pulmonary Sepsis
Pulmonary Ultrastructure

INTRODUCTION:

The appearance of the Adult Respiratory Distress Syndrome (ARDS) in critically ill patients is a well known phenomenon. It could be the result of congestive heart failure, oxygen toxicity, pulmonary emboli, fluid overload, or sepsis. Fluid overload and sepsis have received the most attention.

Sepsis appears to have the strongest association with ARDS. For example, most instances of ARDS are preceeded by a septic event.[1] However, it is not known how sepsis might lead to ARDS. The possibilities include pulmonary tissue damage by entrapped white cells, pulmonary microthrombosis, and surfactant destruction.

Little has been published concerning the pulmonary ultrastructural changes related to septicemia. Those reports that have been published have been limited to short term studies following bolus injection of endotoxin or live bacteria.[2,3] The pulmonary consequences of a more prolonged and endogenous septicemia are unknown. The object of this report is to describe the pulmonary ultrastructural changes seen in rats subjected to 48 hours of lethal endogenous septicemia.

MATERIALS AND METHODS:

Sepsis Model: Sixty male king rats weighing between 175 - 200 grams were used in this study. In thirty rats, the right colon was exposed through a paramedian incision under intraperitoneal nembutal anesthesia (0.04 mg/kg). The lower

one-half of the cecum was ligated with a 000 silk suture and then punctured twice with a No. 19 needle. The abdomen was closed with 000 chromic and skin staples. In 30 control animals, the same procedure was carried out except that the cecum was left undisturbed.

Lung Studies: Forty-eight hours after operation, 5 surviving rats from both the control and experimental groups were killed by decapitation. The lungs were fixed in situ by the method of dual perfusion.[4]

For morphometric analysis random sections of lung tissue from both control and septic rats were quantitatively analyzed. Using an occular grid (20 mm in diameter disk, 5 mm division in 100 units) one micron the sections were viewed under oil. Thirty-six sections from each of the two groups were assessed for the number of thrombosed capillaries, white cells within the pulmonary interstitium, macrophages per section, and amount of normal lung tissue.

For transmission electron microscopy, the fixed lungs were cut into 1 mm cubes and further fixed in cacodylate buffered 2% osmium tetroxide. Following osmication, the tissues were dehydrated in graded acetone, embedded in araldite and processed for viewing. Some of the sections were stained with alcoholic solution of uranyl acetate and/or 2 percent lead citrate.

For statistical analysis the significance of mean difference was assessed by Analysis of Variants.

RESULTS

All the experimental animals were dead sixty hours after cecal ligation. All the control animals were alive at one week. Cultures obtained at sacrifice in experimental animals from blood and major organs including lungs, heart, and kidney all grew out organisms. The most common were coliforms and bacteroides.

In Table I, the morphometric data is shown. Following sepsis, there is a threefold increase in the number of alveolar macrophages and interstitial white blood cells. In addition, microvascular thrombosis was noted in 5.9 capillaries per sections studied which represents approximately 20% of the capillaries in the section. Also the number of white blood cells seen in the remaining patent capillaries declined by two-thirds. Finally, the amount of normal, non-edematous lung interstitium declined by approximately one-third following sepsis (Figs. 1A & B).

Electron Microscopy: In low power electron micrographs of septic lung tissue, the most striking observations consisted of the presence of myelin figures within the alveolar lumen, an increase in the number of macrophages, degranulation of the dense lamellated bodies of the granular pneumocytes, and plugging of the capillary lumen with various profiles of red blood corpuscles and fibrin. The interstitial space was characterized by patchy areas of edema and extravasation of white blood cells. The thick portion of

the interstitial space showed areas of patchy edema fluid collection and osmiophilic smudging (Figs. 2A & B).

The intracytoplasmic changes within the granular II pneumocyte appeared to be one of the most prominent cytologic alteration. All the granular II pneumocytes studies showed an increased formation of new dense-lamellated bodies and extrusion of these organelles through multiple sites into the alveolar air space. The perinuclear development of immature dense-lamellated bodies with gradual morphologic evolution into empty membrane bound spaces near the cytoplasmic membranes are highlighted in Figs. 3A & B. In contrast, the control granular II pneumocytes showed only apical extrusion. This suggests that these cells were maximally stimulated.

Changes were also observed in the alveolar macrophages. Not only was there an increase in the number of these cells, but their cytoplasm showed various profiles of ingested materials, both particulate and inorganic. These findings were rare in the control state.

The capillary endothelium showed increased numbers of pinocytotic vesicles, some of which contained membrane bound bodies. However, the most striking feature was obliteration of the capillary lumen, either by crenated red blood corpuscles or by a combination of red cell, platelet and fibrin plugs (Fig. 1B). All sections studied showed the capillary lumen in various stages of obliteration.

The observations in the alveolar layer were characterized by changes in the lining layer as well as

within the space itself. The alveolar epithelial cytoplasm showed an increase in the number of pinocytotic vesicles, some of which contained electron opaque granules. Also the surfactant layer showed disruption so that segments within the alveolar lumen was studded with myelin figures of various configurations (Fig. 4). These myelin figures were found in most of the alveolar air spaces.

DISCUSSION:

Details of the cecal ligation model used in this study have been described by Wichterman et al.[5] They showed that rats subjected to ligation plus puncture developed the following characteristics: (1) a highly lethal version of septic shock; (2) survival long enough to affect major organs; (3) positive cultures from blood and tissue. Our findings in this study were similar and therefore we believe this model is useful for studying the pulmonary consequences of septic shock.

One of the elements of the lung seriously affected by the septic insult was the surfactant system. Normally, surfactant is thought to be produced in the dense bodies in the cytoplasm of the granular pneumocytes. These dense bodies migrate from the base of the cell to the apex where the phospholipid is extruded. In septic rats, we found a substantial increase in the number of these organelles as well as multiple aberrant sites of extrusion of the phospholipid into the alveolus. In addition, the normally continuous alveolar surfactant layer in contact with the epithelium had become fragmented and was often seen free in the alveolar lumen. We have previously reported fragmentation and free alveolar surfactant in animals subjected to hemorrhagic shock.[4]

The mechanism by which surfactant production is controlled is unknown. However, beta adrenergic receptors which respond to beta adrenergic stimuli have been found in

isolated granular pneumocytes.[6] Also, we have shown in another report that chemical sympathectomy in neonatal rats resulted in the loss of phospholipid within the dense bodies of the granular pneumocytes.[7] Thus, there is evidence that surfactant production is influenced by the autonomic nervous system. One explanation for the changes seen in the granular pneumocytes in the present study would be increased surfactant production stimulated by the sympathetic nervous system. Sympathetic stimulation could be related either to increased catacholamine levels seen in shock or alternatively the sympathetic nervous system might respond to the presence of damaged alveolar surfactant. Although caution must be used in translating our findings to the clinical setting, our results do suggest two lines of investigation in ARDS: (1) further studies to unravel the relationship of surfactant production and the autonomic nervous system; (2) methods of exogenous replacement of damaged alveolar surfactant in an attempt to minimize alveolar instability.

Another element of the lung affected by septic shock was the pulmonary capillary. Diffuse microthrombosis was seen in all the excised pulmonary specimens. It is possible that this is a diffuse effect related to disseminated intravascular coagulation, a common complication of sepsis. However, this explanation seems unlikely since examination of other tissues in the septic rats failed to reveal microthrombosis. Another possibility relates to pulmonary endothelial damage. Normally endothelial cells contain

proteins, such as factor VIII, which help maintain normal clotting equilibrium in the pulmonary circulation. Carvalho et al[8] recently reported changes in the plasma levels of factor VIII related antigen and factor VIII coagulant in patients with ARDS. Factor VIII related antigen is produced in the endothelial cells while factor VIII coagulant is produced elsewhere. These authors found that factor VIII related antigen was elevated five times above normal levels in patients with ARDS while levels of factor VIII coagulant were only slightly elevated. These results suggest that altered factor VIII may be part of the explanation of pulmonary microthrombosis. A second conclusion is that factor VIII related antigen may be a marker for endothelial damage.

A related issue is the changes noted in the pulmonary endothelial cells of the septic rat. We found no evidence of structural damage, even in the presence of diffuse microthrombosis. The endothelial cell walls were intact and the junction between these cells appeared unchanged. The only major change noted was a substantial increase in the number of pinocytotic vesicles, indicating that these cells retain the capacity to engage in energy consuming processes.

Another finding in our study is the increase in the number of interstitial and alveolar white cells as well as an increase in the number of ingested particles seen in the cytoplasm of these cells. This suggests an increased level of white cell activity in response to sepsis. Yet this

Intracapillary Granulocrit

For determination of pulmonary intracapillary granulocrit, 0.5 micron sections were cut with glass knives using an MT2-B ultramicrotome, and stained with toludine blue with basic fucsin counterstain. Twenty photomicrographs with a primary magnification of 1000 were taken from the 0.5 sections from each animal using a Nikon Microflex AFM microscope.

These negatives were then sandwiched and printed with a positive image computer-generated square-stain test grid as described by Reide and shown in Figure 1.[11]

From this, the intracapillary granulocrit (gct) was calculated for each animal by the equation:

$$\text{Intracapillary gct (\%)} = Pg/Pc$$

Where:

Pg = The number of points failling over granulocytes

Pc = The number of points falling over capillary surfaces

Mean intracapillary granulocrit was then calculated for each experimental group.

The cecum was then replaced and the abdomen closed in one layer with a figure-of-eight 4-0 Dexon suture.

Following anesthesia the mice were returned to the housing facility. The cages were then checked every 8 hours post-operatively and specific animal mortalities were recorded at these intervals. At the conclusion of a four week observation period, the survival study was terminated and mean survival time was calculated for each experimental group.

Lung Studies

Forty additional +C5 mice and 40 -C5 mice were randomized to 4 groups as previously described. Twenty-four hours after anesthesia or CLP, 10 randomly selected animals from each test group were again anesthetized and killed by decapitation. Lung specimens were taken from the right lower lobe of each animal for morphometric analysis of pulmonary intracapillary "granulocrit" (gct) and pulmonary air-blood barrier thickness.

The specimens were individually processed by immediate submersion fixation of 1 mm. tissue cubes in 4% glutaraldehyde solution. Following osmication, fixed tissues were dehydrated in graded alcohols and embedded in Araldite. Five lung tissue blocks from each animal were then randomly selected for morphometric analysis.

The purpose of this study was to evaluate the role of C5 in the pathophysiology of septic lung injury utilizing C5-sufficient and C5-deficient twin mice strains in a lethal sepsis model.

METHODS

Survival Study

Fifty male B10D2 new-line (+C5 mice) and 50 B10D2 old-line mice (-C5 mice) obtained from the Jackson Laboratory were the test animals.[9] The mice were 7-12 weeks in age, weighing between 20 and 30 grams. Throughout the study period the mice were housed three per cage; fresh water and Purina Mouse Chow were continuously available. Room temperature was constant at 37°C with a 12/12 hour lighting schedule.

The mice were randomized to 4 groups: +C5 anesthetic control (N = 25), -C5 anesthetic control (N = 25), +C5 cecal ligation and puncture (N = 25), and -C5 cecal ligation and puncture (N = 25). They were then blinded to strain. Each study animal was anesthetized using 0.6 mg/gm. intraperitoneal Nembutal anesthesia. Control animals received intraperitoneal anesthesia alone. In cecal ligation and puncture (CLP) animals, the cecum and right colon were exposed through a 5 mm. transverse incision in the right lower quadrant. The lower 1/2 of the cecum was ligated with a 4-0 Dexon suture, then punctured twice with a 23-gauge needle.[10]

INTRODUCTION

Since the Adult Respiratory-Distress Syndrome was first described in 1967, there have been many proposed mechanisms for its pathogenesis.[1,2] Recently, interest has focused on the role of complement stimulated granulocytes in this disorder. There is good evidence that non-specific tissue injury results in the release of neutral proteases from damaged normal tissues which cleave the inactive complement components into active chemotactic peptides.[3] In addition, endotoxin may directly activate complement via this same pathway.[4] Complement activation generates C5a which is believed to be the critical activated complement component.

In theory, C5a then stimulates granulocyte production of microbiocidal, reactive oxygen species and promotes granulocyte aggregation and adherence to endothelial cells in the lung.[5,6] The aggregated, C5a-stimulated granulocytes then go on to release their toxic oxygen radicals and to liberate cellular neutral proteases in the pulmonary capillaries. These oxygen radicals have been shown to cause tissue damage in vitro by peroxidation of cellular membranes.[7,8]

KEY WORDS:

Sepsis, Complement, ARDS, Granulocrit, Air-Blood Barrier Thickness

ABSTRACT

One proposed mechanism for the pathogenesis of lung injury in septic animals in that C5a triggers granulocytes to produce and release toxic oxygen radicals which damage cellular membranes in pulmonary capillaries. The authors have investigated the possible role of C5 in septic lung injury utilizing C5-sufficient and C5-deficient twin mice strains. In this lethal sepsis model, mean survival time is increased in C5-deficient mice in comparison to the survival of their C5-sufficient twins. Morphometric results demonstrate a significant increase in intracapillary granulocrit and air-blood barrier thickness twenty-four hours after cecal ligation and puncture in C5-sufficient septic mice. Similarly, mean arterial pO2 is significantly decreased in the C5-sufficient animals. Intracapillary granulocrit, air-blood barrier thickness and arterial PO2 are normal in the septic C5-deficient twins of these animals. These data support the hypothesis that C5 is involved in the pathogenesis of septic lung injury.

THE ROLE OF C5 IN SEPTIC LUNG INJURY

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RUNNING TITLE: SEPTIC LUNG INJURY

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Fig. 3A. Granular II pneumocyte from a lung in sepsis. Note the perinuclear location of a developing dense body (DB) surrounded by a number of mitochondria and a centriole (C). Dense bodies labeled 1, 2 and 3 are in various stages of delamellation (X22,500).

Fig. 3B. The haphazard distribution of the majority of delamellated dense lamellated bodies within the cytoplasm (compare with Fig. 5A & B). Several such bodies appear to have lost most of their content and a confluence has taken place (C). One small immature dense body (DB) occupies a perinuclear position (X48,000).

Fig. 4. The different profiles of extruded dense lamellated bodies within the alveolar air space encountered in septic rats have been mounted for easy morphologic reference (X42,000).

Fig. 2B. Panaromic view of alveolar capillary unit in sepsis. All the major morphologic changes are discernible in this micrograph. Alveolar space is filled with extruded dense bodies from the granular II pneumocyte which are in various stages of disruption (My). A granular II pneumocyte is seen on the right of the micrograph showing various configuration of the dense bodies. One appears in the process of extrusion ( ) away from the apical zone of the pneumocyte. A capillary filled with a RBC can be seen in the left lower corner. Although there is no disruption in the cell to cell contact (D) in the endothelial layer, there is disruption of the basement membrane, notably swelling of basement membrane (Bm). The thicker part of the interstitial space shows presence of edema, however, disruption of collagen fibers are not marked. A myofibroblast with myofilaments (M) can be easily seen. The interstitial space is filled with disrupted membranes (Mc) in various shapes and sizes (X24,000).

LEGENDS:

Fig. 1A. One-micron araldite embedded section of normal rodent lung. Under oil, it is possible to clearly identify the granular pneumocytes (P_1 & P_2), macrophage (M), the alveolar space (AS), the blood vessels (C) and the alveolar and the alveolar capillary membrane (Toluidine blue and basic fuschin stain, X600).

Fig. 1B. One-micron araldite section of a lung in sepsis. The edema in one of the interstitial spaces with swelling of AC membrane, is easily discernible (compare with Fig. 1C). Note aggregation of red blood corpuscles with a capillary lumen. Subtle differences from the granular II pneumocytes in control lung can also be appreciated (X1,000).

Fig. 2A. Cellular components of the normal alveolar capillary unit of rat lung is shown in this micrograph. The relationship of the air space the air space and the capillaries are easily delineated. Three major cell types, epithelial type I, epithelial type II, and the endothelial cells are easily recognized. The distribution of surfactant on the alveolar side, as well as the air-blood barrier (-- --) are easily identifiable (X10,200).

Table I. Morphometric Data
(mean + 1 S.E.M. per section studied; N = 36)

Cell Type	Control*	Sepsis	P Va
Alveolar macrophages	11.5 ± 0.43	33.6 ± 0.96	<.
Interstitial white cells	4.9 ± 0.22	13.9 ± 0.66	<.
Number of thrombosed capillaries	None	5.9 ± 0.27	<.
White cells within the capillary	6.4 ± 0.23	1.8 ± 0.21	<.
Normal lung interstitium (area)	21.7 ± 0.45	13.2 ± 0.34	<.

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response may be paradoxical. There is evidence that these "bacterial killers" may also be lung tissue killers. Yamada et al[9] have shown that endotoxin triggered white cells become adherent to endothelial cell in-vitro and liberate free oxygen radicles leading to cytotoxicity. They, along with others, propose that white cells mediated endothelial damage may be the precipitating event in ARDS. Clinical data supporting the concept of white blood cell entrapment was reported by Powe et al.[10] They injected labeled white cells into patients suffering from ARDS who did not have obvious pulmonary infection. Pulmonary scintigraphy showed an abnormal accumulation of radioactivity. The authors concluded that these findings represented microvascular white cell entrapment. The findings from our study suggest an additional interpretation. Some of the labeled white cells might have migrated into the interstitium and alveolar space and there produced local damage.

Air-Blood Barrier Thickness

For determination of pulmonary air-blood barrier thickness, 60-90 nm. ultra-thin sections were mounted on copper grids on a thin Parlodion coating and the sections were stained for viewing under the electron microscope with 2% uranyl acetate and lead citrate.

Ten photomicrographs with a primary magnification of 5000 were taken from each animal using an Hitachi H-300 microscope. These negatives were then sandwiched and printed together with the previously described square-stair test grid (Figure 2).

From this the arithmatic mean barrier thickness (t) of the air-blood barrier was calculated for each animal by the equation[11]:

$$t = \frac{Z \cdot Ppn}{9\,(I_{ALV} + I_{CAP})}$$

Where:

- t = Barrier thickness (in microns)
- Z = The length of test lines (in mm.)
- Ppn = The number of points falling over pulmonary parenchyma
- I_{ALV} = The number of intersections between Z lines and the alveolar surface of the alveolar-capillary membrane
- I_{CAP} = The number of intersections between Z lines and the capillary surface of the alveolar-capillary membrane

Arterial Blood Gases

Twenty-four hours after anesthesia or operation, the remaining ten randomly selected animals in each test group were again anesthetized using intraperitoneal Nembutal anesthesia and heparinized arterial blood was obtained from the abdominal aorta of each mouse.

Utilizing the operating microscope, the aorta was dissected from the surrounding structures. A 27-gauge teflon catheter which had been flushed with heparinized saline was then inserted into the abdominal aorta and 0.4 cc of arterial blood was removed into a heparinized syringe. Arterial blood gas measurement was then performed on a Corning-120 Blood Gas Analyzer.

Complement (C5) Assay

Blood from the twenty decapitated animals was collected and clotted on ice. It was then centrifuged @ 5000 rpm for 5 minutes in a Fischer Microfuge. The serum was then separated and stored at -70°C for subsequent complement assay. This serum was then analyzed for the presence or absence of C5 by double diffusion gel precipitation on Ouchterlony plates utilizing mouse anti-C5 antibody.

Statistical Analysis

Differences in survival, pulmonary intracapillary granulocrit,

air-blood barrier thickness and arterial pO2 measurements were evaluated by means of an analysis of variance.

RESULTS

Mean survival time is shown in Table 1. There were no deaths in the anesthetic control animals in the 4 week observation period. The mean survival time for the +C5 septic animals was 40 hours. In contrast, mean survival time for -C5 septic animals was 87.8 hours. Statistical significance ($p < .001$) was observed between these 2 groups at each 8 hour time interval throughout the survival study (Figure 3).

Mean intracapillary granulocrit (gct) was 0.87% for +C5 and 0.73% for -C5 anesthetic control animals (Table 1). These differences were not significant. In the septic groups, the +C5 mice had a mean intracapillary gct of 13.53% while the -C5 mice had a mean intracapillary gct of 0.69%. The elevated intracapillary granulocrit in the +C5 septic animals was significantly different from the values for the other 3 groups ($p < .001$), while the intracapillary gct for the -C5 septic mice was not different from either control (Figures 4A and 4B).

Arithmatic mean air-blood barrier thickness is shown in Table 2. Mean air-blood barrier thickness was 2.97 for +C5 and 3.06 for

-C5 anesthetic control animals. These differences were not significant. The +C5 septic mice had a mean air-blood barrier thickness of 7.95 while the value for the -C5 septic mice was 2.94 . While the mean air-blood barrier thickness was significantly increased in the +C5 septic group ($p < .001$), in -C5 septic animals, the air-blood barrier thickness was not statistically different from controls (Figures 4A and 4B).

Mean arterial PO2 was 94.2 torr for +C5 and 95.9 torr for -C5 anesthetic control animals. In the septic groups, +C5 mice had a mean pO2 of 59.2 torr while -C5 mice had a mean pO2 of 85.7 torr. The pO2 seen in the +C5 septic animals was significantly different from the values for the other 3 groups ($p < .001$). The -C5 septic group had a mean arterial pO2 which was not significantly different from either control.

C5 assay by Ouchterlony diffusion was positive in 20/20 +C5 and negative in 20/20 -C5 study animals.

DISCUSSION

The Adult Respiratory Distress Syndrome is a disease of unknown etiology. Considerable experimental evidence has accumulated suggesting that ARDS is triggered by complement activation which is a consequence of sepsis and other predisposing events.

Polymorphonuclear granulocytes produce neutral and acid proteases which have been shown to cleave the inactive complement components into active chemotactic peptides.[3,4] In addition, Ward has shown that endoxotin may directly activate complement via the alternative pathway.[4] Complement activation generates C5a which is believed to be the critical activated complement component. In theory, C5a then stimulates granulocyte production of microbiocidal, reactive oxygen species and promotes granulocyte aggregation in pulmonary capillaries.[5,12,13] These toxic oxygen radicals are known to damage endothelial cells by induction of changes in membrane lipids.[7] Thus, there is indirect evidence that complement activated neutrophils cause the endothelial and interstitial injury characteristics of ARDS.[5,12]

Quantitative morphometric techniques have been used extensively in the estimation of pulmonary diffusion capacity. Weibel, et al. have established that the average thickness of the air-blood barrier can be calculated from the volume/surface ratio of the layer of tissue which forms this barrier.[14] In addition, using morphometric analysis Riede has reported a higher volume density of granulocytes per unit volume of capillary lumen in several patients who died from acute respiratory insufficiency.[11] We have applied these same morphometric techniques for the calculation of pulmonary air-blood barrier thickness and intracapillary granulocrit to a small animal sepsis model of ARDS. This allows

an accurate, quantitative comparison of the amount of interstitial edema and the number of granulocytes in the pulmonary capillaries of animals from different experimental groups. Moreover, because the thickness of the air-blood barrier directly influences pulmonary diffusion, it provides a morphologic means of assessing pulmonary gaseous exchange.[15] The measurement of both pulmonary air-blood barrier thickness and arterial pO2 permits a quantitative study of the relation between lung structure and its gas exchange function in ARDS and other disease states.

Previous investigations on the role of complement in ARDS have employed cobra venom factor and other substrates which are known to activate the complement system.[3,16] It has been difficult in these studies to control for the possibility of a direct effect of these substances on pulmonary capillary integrity. Our model which instead employs C5-sufficient and C5-deficient twin animal strains, offers an opportunity to eliminate exogenous substances from the experimental design. In addition, it provides a study population of identical genetic background with the exception of the variable under study.

Complement components and complement genetic polymorphism have been studied and characterized in a wide variety of animal species.[17] Because of a remarkable similarity between the mouse and human complement systems, the mouse provides an attractive

model for the study of the role of complement in ARDS. The B10D2 old-line mouse is a highly inbred normal mouse developed at the Jackson Laboratory, which differs from its coisogenic twin, the B10D2 new-line mouse only by a small deletion mutation on chromosome 2 in the old-line mouse resulting in a complete deficiency of C5 in these animals.

Since C5a is the first common factor in the common trunk of the complement cascade, the C5-deficient mouse is unable to activate complement by either the classical or alternative pathway, and is unable to generate C5a.[17] If C5a and C5a-induced pulmonary granulocyte aggregation are a necessity in the mechanism of septic lung injury, these should not occur in the C5-deficient mouse. This is supported by our finding of a normal intracapillary granulocrit in the C5-deficient septic animals with a corresponding increase in intracapillary granulocrit in the C5-sufficient septic mice. Similarly, the significantly increased mean survival time of the C5-deficient septic animals in comparison to the survival of their C5-sufficient twins, suggests that the presence of C5 may be a detriment to the survival of septic animals.

Although the mean survival time is significantly prolonged in the C5-deficient septic animals, these animals do also eventually die. It is not clear whether the C5-deficient animals succumb to

overwhelming sepsis without evidence of ARDS, or whether instead, these animals simply develop lung injury later in their septic course. In order to clarify this problem, it would be helpful to repeat each of the morphometric parameters in the C5-deficient animals 48 and 72 hours after the lethal septic insult.

If the C5-deficient animals, do, in fact, ultimately develop the pathophysiologic features of ARDS this would not be inconsistant with the proposed pathogenetic mechanism of lung injury. With endotoxin activation of the alternative complement pathway, C3a production continues in the C5-deficient animals. This potent anaphlotoxin may cause the release of vasoactive peptides which will in turn cause cellular permeability changes.[3] C3a has been shown to cause mast cell and basophil degranulation with release of the vasoactive substances: histamine and serotonin.[4] In addition, endotoxin may directly trigger granulocytes to release their proteolytic enzymes resulting in pulmonary architectural disruption. Finally, the C5-deficient mouse may even possess some other complement-like pathway unique to these animals which has not yet been elucidated.

Mean pulmonary air-blood barrier thickness is significantly increased in the C5-sufficient septic animals. This is associated with a significantly decreased mean arterial pO2 in these animals. The finding of a normal air-blood barrier

thickness and arterial pO2 in the septic C5-deficient animals lends further support for the hypothesis that C5 may be an important factor in the mechanism of lung injury in sepsis. One explanation for the significantly decreased mean arterial pO2 seen in the C5-sufficient septic mice is that this is purely the result of impaired gas exchange through the markedly thickened pulmonary air-blood barrier found in these animals. Another possibility is that the decline in arterial pO2 seen in the C5-sufficient septic animals is a consequence of a diminution in the cardiac output of these animals, perhaps on the basis of volume depletion or early multiple organ failure. Such a reduction in blood flow through the lungs could result in a mismatch between ventilation and perfusion.

While the have not yet prospectively studied granulocyte aggregation in other organs as we have in the lung, a preliminary retrospective review of liver biopsies from the various experimental groups has raised the question of whether complement activation of granulocytes occurs not only in pulmonary capillaries, but also in other organs as well.[7] If further studies confirm that this does in fact occur, it suggests that C5 might also play a role in the pathogenesis of the syndrome of multiple organ failure which occurs in animals with intra-abdominal sepsis.[7,18]

While extrapolation from a rodent model to the human situation is problematic, these data support the hypothesis that complement activated neutrophils play a similar role in the pathogenesis of the endothelial and interstitial injury characteristic of the human Adult Respiratory-Distress Syndrome.[19,20]

ILLUSTRATION LEGENDS

Figure 1: Determination of pulmonary intracapillary granulocrit with a positive image, square-stair test grid. The relationship of the alveolar and capillary (C) spaces are shown. Capillaries contain endothelial lining cells (En), erythrocytes and polymorphonuclear granulocytes.

Figure 2: Section of the septum between an alveolus (ALV) and capillary (C). Note the considerable variation in the thickness of the air-blood barrier with thin and thicker regions. The barrier is composed of an epithelial (Ep), an endothelial (En) and an interstitial layer. The arithmatic mean thickness of this barrier can be determined morphometrically. Normal mouse lung x 5000.

Figure 3: Cumulative actuarial survival of animals in the C5-sufficient and C5-deficient septic groups is shown.

Figures 4A & 4B: Sections of lung from +C5 septic (Figure 4A) and -C5 septic (Figure 4B) mice. Note the increased numbers of granulocytes in the pulmonary capillary of the +C5 animal as well as the marked edema of the alveolar-capillary septum. Also illustrated is the increased thickness of the air-blood barrier in the +C5 septic mouse.

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TABLE 1

Mean Survival Time and Intracapillary Granulocrit in +C5 and -C5 Mice

	ANESTHETIC CONTROL		CECAL LIGATION AND PUNCTURE	
	+C5	-C5	+C5	-C5
Mean Survival Time (Hrs.)	-	-	40 ± 3.8*	87.8 ± 4.1
Mean Intracapillary Gct. (%)	0.87 ± 0.27	0.73 ± 0.11	13.53 ± 0.43*	0.69 ± 0.12

*$p < .001$ by analysis of variance

Results are expressed as mean ± S.E.M.

TABLE 2

Mean Air Blood Barrier Thickness and Arterial pO2 in +C5 and -C5 Mice

	ANESTHETIC CONTROL		CECAL LIGATION AND PUNCTURE	
	+C5	-C5	+C5	-C5
Mean Air-Blood Barrier Thickness (Microns)	2.97 ± .04	3.06 ± .14	7.95 ± 20*	2.94 ± .16
Mean Arterial pO2 (Torr)	94.2 ± 2.29	95.9 ± 2.8	59.2 ± 4.44*	85.7 ± 3.61

*$p < .001$ by analysis of variance

Results are expressed as mean ± S.E.M.

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This study describes changes in pulmonary ultrastructure in rodents following cecal-ligation-and-puncture-induced sepsis. Lungs removed from rats 48 hours post-sepsis were studied by electron microscopy. Findings were: diffuse capillary thrombosis, hyperactive appearing granular pneumocytes plus free alveolar surfactant, increased number of interstitial white blood cells and alveolar macrophages plus decreased number of white blood cells within capillaries. Control animals receiving laparotomies without cecal ligation or puncture showed none of these changes.

The possible role of C5 in producing ultrastructural changes in the lungs of septic animals was investigated using C5-sufficient (C5+) and C5-deficient

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(C5-) twin mice strains subjected to cecal ligation and puncture. Mean survival time for C5- septic animals was significantly longer than for C5+ mice. Arterial pO2 24 hours post sepsis was significantly lower in C5+ animals than in either C5- septic animals or C5+ or C5- controls. Ultrastructural changes in C5+ septic animal lung included increases in intracapillary granulocrit and air-blood barrier thickness. Lung ultrastructure was normal in C5- septic animals and all controls.

These results suggest that septic shock produces injuries to all compartments of the lung and that C5 is a crucial component in the pathogenesis of septic lung.

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